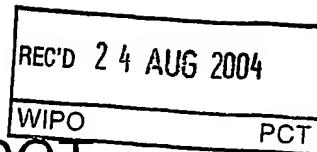


PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY



PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/CA2004/000453

International filing date (day/month/year)
25.03.2004

Priority date (day/month/year)
26.03.2003

International Patent Classification (IPC) or both national classification and IPC
C07K7/00, C12Q1/37

Applicant
BIOMEPE INC.

1. This opinion contains indications relating to the following items:

- Box No. I Basis of the opinion
- Box No. II Priority
- Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- Box No. IV Lack of unity of invention
- Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- Box No. VI Certain documents cited
- Box No. VII Certain defects in the international application
- Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



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Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 a sequence listing
 table(s) related to the sequence listing
 - b. format of material:
 in written format
 in computer readable form
 - c. time of filing/furnishing:
 contained in the international application as filed.
 filed together with the international application in computer readable form.
 furnished subsequently to this Authority for the purposes of search.
3. In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
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Box No. II Priority

1. The following document has not been furnished:

- copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
 translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

- the entire international application,
 claims Nos. 1-48 (partially)

because:

- the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
 the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 no international search report has been established for the whole application or for said claims Nos. 1-48 (partially)
 the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:
the written form has not been furnished
 does not comply with the standard
the computer readable form has not been furnished
 does not comply with the standard
 the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-bis of the Administrative Instructions.
 See separate sheet for further details

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

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**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or
industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	5,7,9-30, 35, 37, 39-48
	No:	Claims	1-4, 6, 8, 31-34, 36, 38
Inventive step (IS)	Yes:	Claims	5,7,9-30,35,37,39-48
	No:	Claims	1-4,6,8,31-34,36,38

Industrial applicability (IA) Yes: Claims 1-48
 No: Claims

2. Citations and explanations

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The subject-matter of claims 1 and 31 relates to a fluorogenic PHEX substrate comprising, respectively, a peptide unit including the sequence P2-P1-P1'-P2' and P3-P2-P1-P1'-P2'-P3' wherein P2 can be any amino acid residue, P1 can be any amino acid residue except an isoleucine, a valine, or a histidine, P1' a glutamic acid or an aspartic acid and P2' being any amino acid residue except a leucine, a proline or a glycine residue.

The said claims encompass so many sequences (over 3000000 sequences found in the STN database) that the requirements of conciseness (Art. 6 PCT) are not met. As it would be an undue burden to search all of the said sequences, the search has been restricted to those sequences which are clearly identified within the filed sequence listing, namely SEQ ID NO:2 to SEQ ID NO:7 and SEQ ID NO:10 to SEQ ID NO:32.

Consequently, an opinion with regard to novelty, inventive step and industrial applicability of claims 1-48 is only given with regard to the above sequences which have been searched.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

- D1: SONG EUN-SUK ET AL: "Analysis of the subsite specificity of rat insulin using fluorogenic peptide substrates" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 2, 12 January 2001 (2001-01-12), pages 1152-1155,
- D2: MELDAL MORTEN: "Multiple column synthesis of quenched solid-phase bound fluorogenic substrates for characterization of endoprotease specificity" METHODS (ORLANDO), vol. 6, no. 4, 1994, pages 417-424,
- D3: WO 00/50580 A (BOILEAU GUY ; UNIV MONTREAL (CA); CRINE PHILIPPE (CA)) 31 August 2000 (2000-08-31)

2. The subject-matter of claim 1 is not new (Art. 33(2) PCT). Document D1 discloses the fluorogenic peptide Abz-GGFDRKVGQ-EDDnp, the said peptide having a fluorophore unit and a quencher unit and falls within the scope of SEQ ID:NO 4 to SEQ ID NO: 7 and SEQ ID No:10, thus being a PHEX substrate. Similarly, document D2 disclose a fluorogenic peptide having the sequence Y(NO₂)IGPLEMK(ABz), falling within the scope of SEQ ID:NO 4 to SEQ ID NO: 7 and SEQ ID No:11, thus being a PHEX substrate.

For the same reasons, the subject-matter of independent claim 31 and dependent claims 2-4, 6, 8, 32-34, 36 and 38 is also not new (Art. 33(2) PCT).

3. As the particular combination of features of dependent claim 5 (with the restriction made under above Item III) is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

Moreover, the subject-matter of the said claim involves an inventive step in the sense of Art. 33(3) PCT.

Documents D1 and D2 disclose fluorogenic peptides falling within the scope of claim 1 and thus having the intrinsic property of being substrate of PHEX.

The subject-matter of claim 5 differs from D1/D2 in that other fluorogenic PHEX substrates are disclosed.

The technical problem to solve was therefore to provide further fluorogenic PHEX substrates.

To solve the said problem, the applicant has synthesized peptide libraries and used positional scanning synthetic combinatorial libraries to identify substrate specificity. By doing so, the applicant has identified some amino acid residues preferred by PHEX in substrate positions P2 to P2'. Documents D1 and D2 do not disclose any amino acid requirements as the said documents are not concerned with PHEX.

Document D3 discloses that PTHrP107-139 is a substrate of PHEX. The said document further discloses that the said peptide can be used to design and synthesize internally quenched fluorescent substrates for PHEX (p. 22, "assay for the activity of PHEX" and p.29-30, examples II and III). However, D3 does not disclose any peptide sequence and amino acid requirements.

Thus, as the sequences disclosed in claim 5 are neither disclosed nor suggested in the cited prior art, the subject-matter of the said claim 5 (with the above restriction mentioned under Item III) is inventive (Art. 33(3) PCT).

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AUTHORITY (SEPARATE SHEET)**

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For the same reasons, the subject-matter of dependent claim 7, 9, 35 and 37 are also new (Art. 33(2) PCT) and inventive (Art. 33(3) PCT).

4. As the particular combination of features of independent claims 10, 21, 39 and 40 (with the restriction mentioned under Item III) is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

Moreover, although documents D1 and D2 disclose fluorogenic peptides having the intrinsic property of being substrate of PHEX (see above point 3), the said documents are not concerned with the PHEX enzyme. Thus, the said documents do not give any incentive to use the said peptides in methods concerning PHEX. Furthermore, the skilled man would not consider the said documents when studying PHEX.

Thus, the subject-matter of claims 10, 21, 39 and 40, concerning various uses related to PHEX of the peptides disclosed in claims 1 and 31, involves an inventive step in the sense of Art. 33(3) PCT.